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Biotechnological potential of marine sponges and their associated bacteria as producers of new pharmaceuticals (Part II)

The supply problem

The examples of sponge-derived compounds in advanced stages of clinical trials presented in part I of this article emphasize the potential of sponges as auspicious source for drugs against various human diseases.

However, compared to the vast number of over 4000 compounds isolated from sponges during the last three decades, the number of sponge-derived drugs that have already entered the market is surprisingly small. There are two major reasons for this phenomenon: One is the extremely long time frame involved in the process of drug development. For instance, to develop the famous anticancer drug Taxol® from its initial description in the yew tree to its approval as a commercial pharmaceutical took over 20 years. The presented examples of sponge-derived compounds in clinical trials, this long time frame is by far no exception. Thus, as many interesting compounds were initially reported already in the 1980s and early 1990s, there is hope that within the following years the number of commercially available "marine drugs" will considerably increase.

The second reason for the comparatively small number of sponge-derived drugs that are so far on the market is the fact that most pharmaceutically interesting natural products are available only in minute amounts from their natural sources, as they are present in sponge tissue in very low quantities. For structure elucidation, pharmaceutical and pharmacological assays and later on for clinical trials, however, considerable quantities of these compounds are needed. The lack of material is in fact the major limiting factor for the development of sponge-derived compounds to commercial drugs. Moreover, it has to be asked where the material for drug production should come from, in case the agent should really make it to the market. This problem is vividly illustrated by the example of the sponge-derived halichondrins (see part I). An annual need between 1 and 5 kg per year is estimated if these compounds should once be commercially available as anticancer drugs. If tissue of the sponge *Lissodendoryx* sp. was the only source of this material, this would mean harvesting 3,000–16,000 metric tonnes of sponge biomass per year. It is obvious that such large amounts could neither be taken from nature without risking extinction of the respective source species, nor could such an approach be at all economically feasible. Therefore, alternative strategies are needed to make sponge compounds better accessible for drug development.

One solution may be chemical synthesis. Usually, once a new natural product has been discovered and there is proof for highly interesting pharmacological properties, many chemical labs all over the world quickly start attempts to synthesize it. However, natural products are often characterized by highly complex molecules, rendering their synthesis very labor intensive and thereby economically not feasible (especially when their fate in further clinical trials is precarious). Moreover, it has been shown that sometimes the synthetic products feature different pharmaceutical properties compared to their natural counterparts, even if all physicochemical measurements for both compounds are identical.

Another possibility to provide higher amounts of a pharmaceutical relevant compound derived from marine invertebrates is mariculture, i.e., in-the-sea cultivation of the source organism. This has proven fairly successful for the tunicate *Ecteinascidia turbinata*, which produces the anticancer drug "Yondelis®" that is now in Phase III clinical trials conducted by the Spanish company PharmaMar and for the bryozoan *Bugula neritina*, source of the anticancer agent bryostatin 1, which is in Phase II clinical trials by GPC Biotech (Mendola 2000). The example of the abovementioned deep-water sponge *Lissodendoryx* sp. that still produces halichondrins when cultivated in shallow-water is promising as well. The obtained compound yields, however, are still far from those that will be needed once one of these compounds has finally entered the market. Moreover, mariculture does not afford complete

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control of environmental parameters entailing the production outcome to be unpredictable to a certain extent (Osinga et al. 1999). Development of closed-system bioreactors for spong tissue cultivation could be a solution for the latter problem and is, therefore, a challenging opportunity for marine bioprocess engineers (Belarbi et al. 2003).

Sponge-associated microorganisms – a possible solution to the supply problem

Sponges, as well as most other marine invertebrates known to yield pharmaceutically interesting metabolites, often harbour microorganisms in their tissues. In many sponge species, the associated bacterial communities account for over 40% of the biomass of their hosts, while in certain cases this value can even reach almost 60% (Vacelet 1975, Willenz and Hartmann 1989, Friedrich et al. 1999). The microbial numbers in sponge tissues frequently exceed those of the surrounding seawater by two to four orders of magnitude (Friedrich et al. 2001, Webster et al. 2001).

Interestingly, the compositions of the bacterial communities within sponges are often considerably different from those found in any other natural environment (Hentschel et al. 2002, Taylor et al. 2004, 2005, Olson and McCarthy 2005). Moreover, in several studies on sponge-associated bacteria, the microbial assemblages proved to be extraordinarily stable even under altered environmental conditions of the sponge (Maldonado and Young 1998, Friedrich et al. 2001, Thoms et al. 2003a, Taylor et al. 2005). The sponge tissue (called mesohyl) seems to provide a unique ecological niche for very particular bacterial species. Therefore, sponge-associated bacterial communities have become an exciting target for microbiological research, revealing new bacterial species and even new bacterial phyla with unprecedented metabolic properties (e.g., Preston et al. 1996, Hentschel et al. 2001, Ahn et al. 2003, Pimentel-Elardo et al. 2003, Fieseler et al. 2004).

In recent years, more and more evidence has accumulated that many of the alleged "sponge chemicals" are not produced by the sponges themselves, but are in fact products of the metabolic activities of bacteria living in the sponge tissue (Faulkner et al. 2000, Haygood et al. 2000, Hildebrand et al. 2004). In many cases there is circumstantial evidence for this supposition by distinct structural similarities between compounds extracted from sponge tissue and known products of bacterial biosynthesis. In some cases, it has already been proven that metabolites earlier ascribed to sponges are indeed of bacterial origin (Unson and Faulkner 1993, Bewley et al. 1996, Schmidt et al. 2000). In most cases, however, the final proof is still lacking. Given the fact that most of the metabolic and biochemical diversity of life does reside in microorganisms it would not be surprising if bacteria would considerably contribute to the astounding arsenal of secondary metabolites found in sponges. This, in turn, could be a solution for the aforementioned supply problem: If successfully cultivated outside the sponge tissue, these functional bacteria (i.e., the bacteria responsible for the metabolite production) could be used as biosynthesizers of the respective compounds. As pharmaceutical companies already have a great deal of experience in fermentation of bacteria, this approach could potentially facilitate natural product production at an industrial scale sufficient to supply the needs for drug development as well as to provide enough material for the market (Faulkner 2000b, Haygood et al. 2000, Newman and Cragg 2004). Even in cases where cultivation of a functional sponge-derived bacterium proofs difficult or impossible, once the biosynthetic genes in the bacterial genome are discovered, they could be cloned and reconstituted in heterologous host bacteria easier amenable to large-scale cultivation, allowing gene expression and compound production (Haygood et al. 2000, Hildebrand et al. 2004).

However, bacterial communities in sponges are often extremely complex. They are an intricate assemblage of bacteria able to persist in the sponge mesohyl over prolonged periods of time (even for the entire life span of the sponge), as well as of transient bacteria species that emerge only briefly when filtered from the seawater, but disappear immediately as soon as they vanish from the surrounding environment (Hentschel et al. 2002, Thoms et al. 2003a, Taylor et al. 2004). All intermediate stages between these two extremes exist in sponges too. While this presents opportunities for interactions that lead to the observed great diversity of natural products in sponges, it also complicates microbiological analysis substantially. Usually microbiological studies on the sponge-associated bacterial communities provide only a snapshot of the complex bacteria assemblages in sponges, making it highly intricate to distinguish between true symbionts constantly associated with the sponges and transient bacteria that rather serve as food for the sponge cells. However, the metabolic activities of bacteria species permanently associated with sponges are more likely to have a considerable effect on the natural product profiles of their hosts.

When sponge-associated bacterial communities are used to inoculate cultivation media, the cultivated bacterial assemblage usually differs substantially from that found in the sponge tissue (Amann et al. 1995, Connon and Giovannoni 2000, Kaeberlein et al. 2002, Olson and McCarthy 2005). This can be explained by the fact that so far less than 1% of sponge-associated bacterial species are cultivable outside their hosts, representing the small fraction of the original microbial community laboratory cultures typically are composed of (Friedrich et al. 2001, Webster and Hill 2001). As the remaining 99% are usually superseded by few faster-growing species in cultivation, it is extremely difficult to access them for investigations on their biosynthetic properties. Moreover, even if interesting sponge-derived bacteria can be cultivated on commercially available laboratory media, this often results in substantially altered metabolic activities without the desired compound being produced (Hugenholtz et al. 1998, Cragg and Newman 2001). The matter is a vicious circle: The fact that many sponge bacteria for the most part are so far uncultivable outside the sponge tissue hampers the discovery of functional symbionts in the complex sponge-associated microbial communities. As long as these symbionts can't be determined, it is not possible to focus research efforts on them to improve cultivation conditions or to transfer biosynthetic genes to cultivable bacteria.

To break this vicious circle, future research should be directed towards the following objectives:

1) Circumstantial evidence for bacterial biosynthesis of "sponge" natural products has to be collected, thereby identifying appropriate sponge/bacteria systems for further symbiosis research. This includes comparison of the chemical structures of compounds found in sponges with metabolites known to be produced by bacteria, as well as fractionation of sponge tissue, separating bacteria from sponge cells in order to determine which of the two fractions yields the respective compounds.
2) True symbionts have to be distinguished from transient bacteria within the bacterial communities of sponges in order to confine the complex communities to candidates that are likely to produce the desired compounds.
3) Current cultivation-independent biomolecular techniques have to be applied to correlate the presence of certain bacterial species with the presence of pharmaceutically interesting compounds. For example cultivation-independent methods can be used to compare microbial communities of different sponge species yielding identical secondary metabolites in order to determine whether they share certain bacterial species. Another approach is to artificially affect the bacterial community of a sponge and to monitor the resulting effects with biomolecular techniques, while at the same time observing changes in the natural products profile by chemical analysis.
4) New cultivation techniques have to be designed, aiming to imitate the natural conditions in the sponge tissue in order to increase the percentage of sponge-derived bacteria amenable for laboratory cultivation without altering their biosynthetic properties.

A lot of efforts are presently made in this field and big hope lies on this research, as once the biotechnological potential of sponge-derived bacteria becomes utilizable, this could quickly and substantially increase the number of FDA approved marine drugs against various human diseases.

Legal considerations for marine bioprospecting

The monetary value of drugs from the sea, as well as other marine biotechnology industries (aquaculture, food additives, cosmetics, antifoulants, agrochemicals, dyes, enzymes for industrial applications) has been estimated at US $100 billion in worldwide sales in 2000 (Gorina-Ysern 2003). Given the enormous potential of this industry, revenues are likely to increase steadily over the coming years. Developed countries - often relatively poor in biological resources - seek to access and bioprospect biodiversity-rich undeveloped countries, which often don't have the industrial, financial and scientific capabilities to explore and bioprospect their resources. Bioprospecting is referred to as the collection of biological material for the screening and investigation of exploitable compounds, genetic information or design (biomimicry). How to access biological materials for bioprospecting in foreign countries has caused a broad discussion about the ownership of biological materials in general. There are two conventions that have to be considered when discussing bioprospecting of marine organisms:

1) The United Nations Convention on Biological Diversity (Biodiversity Convention, 1992)
2) The United Nations Convention on the Law of the Sea (UNCLOS, 1982)

Both conventions give clear ownership of biological resources to the states in which they occur. The Biodiversity Convention states that "genetic

resources, organisms or parts thereof, populations, or any other biotic component(s) of ecosystems with actual or potential use or value for humanity" are biological resources. As such they are covered under international law, which gives the states the "sovereign right to exploit their own resources...". Besides regulating the ownership, the Biodiversity Convention also guides on how to protect, regulate, manage and ensure sustainable use of their biological resources. The UNCLOS convention regulates how much marine area (and the natural resources therein) is under the jurisdiction of coastal states. Coastal states have as such sovereignty over their territorial water, which is the 12 Mile Zone (12 nautical miles from their shorelines, UNCLOS Article 3). In addition they have sovereignty over the Exclusive Economic Zone (EEZ), which ranges 200 nautical miles from their shores. "States have the sovereign right to exploit their natural resources pursuant to their environmental policies and in accordance with their duty to protect and preserve the marine environment" (Article 193). On the other hand access to biological resources for research purposes is explicitly supported by UNCLOS Part XIII, the Marine Scientific Research Provisions: Article 238 under UNCLOS Part XIII gives all states, whether they have their own territorial waters or not, the right to conduct marine scientific research with the restriction that it can only be conducted "...with the expressed consent of and under the conditions set forth by the coastal state". Therefore, within the EEZ, coastal states have the right to give or refuse their consent to marine scientific research and maintain the right to regulate its conduct (see the review by Farrier and Tucker, 2001, for further details).

In summary, while the intentions from the Biodiversity Convention and UNCLOS are somewhat different, both provide coastal states with the legal right to grant or deny access to biological resources and to bargain and define the terms of collection rights. While both conventions are beneficial for coastal countries, these countries have to consider that too many restrictions and monetary compensation *a priori* to any research efforts might discourage researchers and organizations altogether from engaging in such endeavors. High demands for monetary compensation have been fuelled by unrealistic expectations of future royalties resulting from the collection and screening of biological materials in their coastal waters and ultimately the development of commercial drugs. However, drug development is a high-risk undertaking, which usually takes 10 to 20 years and an investment in access of $ 250 millions from the point of discovery to the marketable drug. Therefore, a better strategy for source countries might be to adapt a more open access policy, where both parties (source country and researchers) engage in a broader collaboration. A good example is the bioprospecting agreement form the U.S. National Cancer institute (NCI). NCI tries to increase the capacity of source countries by actively engaging them in drug discovery and development. Their attempt is to "make sincere efforts to transfer knowledge, expertise and technology related to drug discovery and development...subject to the provision of mutually acceptable guarantees for the protection of intellectual property associated with any patented technology."

Conclusions

The marine environment provides a vast repertoire of chemical structures that frequently have no comparable equivalent in terrestrial organisms and often possess an exciting potential as drugs against various human diseases. The most fruitful sources in this respect are marine sponges. More natural products possessing potent bioactivities and unprecedented molecular architectures have been reported from these animals than from any other marine invertebrate phylum. Several of these compounds are now being tested in clinical trials as drugs against various diseases. However, tapping the full potential of this source is so far severely hampered by the lack of supply of the respective compounds for drug development. Big hope lies in the supposition that in many cases microorganisms are the true producers of the pharmaceutically relevant compounds found in sponge tissue. These functional bacteria could be fermented by pharmaceutical companies for large-scale production of the respective substances. Future research, therefore, has to be directed towards the identification of bacterial species within the complex microbial communities that are responsible for metabolite biosynthesis. Subsequently, adequate cultivation techniques for the sponge bacteria, or alternatively, ways to transfer biosynthetic genes from these microbes into bacteria that are easier amenable to cultivation have to be developed in order to facilitate industrial-scale compound production. Once these challenges are overcome, the vast number of sponge-derived metabolites already known, as well as the even greater number of compounds in sponge tissue that are yet to be discovered, could soon become a very important source of drugs against cancer, malaria, microbial infections and many other human diseases.

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